POLARIZATION, KINETOCHORE MOVEMENTS, AND BIVALENT STRUCTURE IN THE MEIOSIS OF MALE MANTIDS

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Introduction

The pioneer studies of mantid cytology of Giardina (1897), Oguma (1921) and King (1931) were concerned primarily in establishing the chromosome complement and the existence of the X_1X_2Y, ♂, and $X_1X_1X_2X_2$, ♀, sex chromosome mechanism. The meiotic bivalents were found to conform to the usual orthopteran types and few or no data were given on the prophase behavior of any except the sex chromosomes. The later papers of Williams (1938) and Erazi (1940), while in general supporting the earlier conclusions, are based on inadequate material and analysis. Erazi's report of an XY or XX sex chromosome complement in the male of *Empusa pennicornis* should be checked on more extensive material.

The recent studies of White (1938, 1941) have resulted not only in his beautiful analysis of the compound sex chromosome complex, and the discovery of an XO ♂, XX ♀ sex chromosome mechanism in many species, but have also disclosed in the meiosis of male mantids three other problems of major cytological interest. First is the nature of the bouquet stage. The occurrence of two separate polarizations of the chromosomes at different periods of the prophase,—the second of which takes place at pachytene—offers an exceptionally favorable opportunity for an analysis of bouquet formation. The second problem is posed by the complicated series of kinetochore movements. In most mantid species the spindle forms in late pachytene, whereupon homologous kinetochores move suddenly apart toward opposite poles forcibly stretching open the bivalents in the developing spindle. This unique movement is followed by the re-approach of the homologous kinetochores as the chromosomes again contract; only then do the bivalents move to their final position in the metaphase plate, following which the real anaphasic movement is initiated. The third problem—the relation of chiasmata to bivalent structure—is presented by the variation among different species in the form of the bivalents during the stretching process and at first metaphase. In Callimantis, where the stretch phenomenon is absent, the bivalents retain the parallel association of their homologous chromosomes, except for a localized separation at the kinetochore region, until anaphase. The cytological evidence for the complete absence of visible chiasmata at all stages in these bivalents is unequivocally clear (White, 1938; Hughes-Schrader, 1943). In all the other species investigated by White (1941) the pre-metaphase stretching of the bivalents discloses terminal connections between their homologous chromosomes suggestive of previous chiasma formation.

Obviously the amazing range of chromosome and more specifically kinetochore movement, and in type of bivalent structure indicated above afford data which bear significantly on many problems of the mitotic mechanism. Until an experimental analysis becomes possible, the comparative study of these phenomena in many related species offers the best approach to the problems they pose. The present report covers five species, in one of which are found two distinct types of prophase behavior correlated with different geographic distribution. The cytological data are presented separately for each species,—followed by a comparative study of the problems outlined above as clarified by the variations presented in the different species.

Material and Methods

Males of the following four species were collected on Barro Colorado Island, Panama Canal Zone, during December of 1939 and 1940: *Liturgousa annulipes* Serv.,—six pre-adult nymphs, two adults; *Stagmomantis carolina* Johann,—two adults; *Angela guianensis* Rehn,—one adult; and *Choeradodis rhombicollis* Latreille,—two adults. *Stagmomantis carolina* was also collected in the region of Onancock, Virginia, in July and August 1941 and 1942; fifteen pre-adult and two adult males were used in the present study. Also from Onancock is the material of *Paratenodera sinensis* Saus.,—comprising seven pre-adult males. My experience confirms White's (1941) report that the pre-adult male offers the most extensive range of stages in spermatogenesis. It is a pleasure to thank Dr. James A. G. Rehn of the Academy of Natural Sciences of Philadelphia for the identification of all of the specimens, with the exception of the Paratenodera.

The fixatives of Sanfelice, and Bouin as modified by Allen and Bauer, were used exclusively. Material was sectioned at from 8 to 12 micra and variously stained in iron haematoxylin, Smith's modification of Newton's gentian violet, La Cour's chromic acid gentian violet, and Feulgen. Counterstaining with erythrosin after gentian violet and with light green after Feulgen was useful for spindle and nuclear membrane differentiation.

Cytological Data

The three main problems—(1) the second polarization or bouquet stage, (2) kinetochore movements, and (3) bivalent structure and chiasmata, will be considered separately under the different species. Early prophase, prior to pachytene, is remarkably uniform in all and a few words here will apply to all the species studied. Leptotene and zygotene stages fix and stain poorly, and no detailed analysis of them has been attempted. The major features of chromosome behavior can, however, be established. Leptotene is characterized by a typical bouquet formation in which the ends of all the autosomes are aggregated on a restricted region of the nuclear membrane immediately underlying the division center,—while the bodies of the chromosomes extend through the nucleus in loops. The sex chromosomes are also polarized. In Liturgousa and Angela the single X undergoes a typical conflexion and its closely appressed ends are aggregated with those of the autosomes. The behavior of the multiple sex chromosomes of Stagmomantis, Choeradodis, and Paratenodera is less open to analysis, but in Paratenodera at least it can be shown that the ends of all three sex chromosomes are involved in the polarization. The bouquet formation persists through zygotene and terminates at different times of the prophase in the different species, but usually during early pachytene. By this time the division center is no longer visibly differentiated from the general cytoplasm.

Stagmomantis carolina *from Virginia*

The diploid complement of the male of this species is 27,—12 pairs of autosomes plus X_1, X_2, and Y. The chromosomes are figured and described by King (1931); I shall therefore confine my account to those aspects of the meiotic prophase bearing on the problems outlined.

I. Second polarization

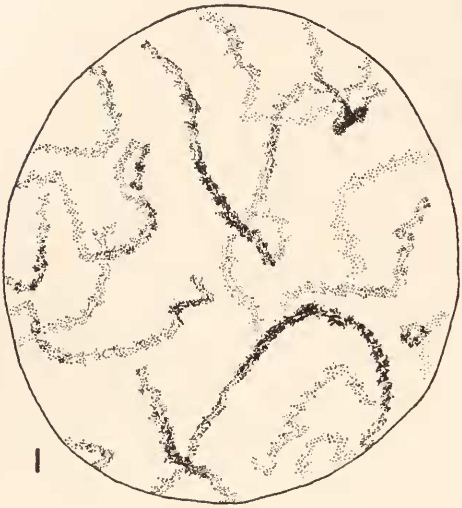
With the cessation of polarization in early pachytene the chromosome ends assume a random distribution in the nucleus, and a completely unoriented stage ensues (Fig. 1). By mid pachytene the division centers again become visible as asters form around them. Thereupon the chromosome ends once more orient actively and aggregate on the nuclear membrane underlying the centers. For the second time, therefore, in this prophase, a bouquet formation is brought about. Two division centers are now involved, however, and this results in variation in the pattern of polarization. Both ends of a bivalent may move to the same pole, or one end may go to each pole stretching the body of the bivalent between them. A double bouquet results—with the ends of the bivalents variously distributed at the two centers (Figs. 2 and 3). The centers are usually already on opposite sides of the nucleus when they first become visible, but occasionally the timing varies and the two poles may lie no more than 90° apart. In such cases, as the centers continue to move toward opposite sides of the nucleus, the grouped chromosome ends follow them on the inside of the membrane, for by late pachytene the two bouquets are invariably some 180° apart. [This precocious activity of the centers and consequent shifting of the aggregated chromosome ends is more common in the Barro Colorado form of Stagmomantis and is illustrated in Figures 12 and 13.] Such a sliding movement of the chromosome ends along the membrane suggests that no real fusion or firm cementing of the two is involved,—although after appropriate staining it is possible to demonstrate that the terminal chromomeres of each bivalent are closely appressed against the nuclear membrane (Fig. 4). The attenuation of the ends of the bivalents might suggest that they are under tension,—but since it is equally apparent in bivalents looped at one pole as in those stretched between two, this impression is misleading. The apparent attenuation more probably stems from the fact that as the gyres of the chromosomes increase in diameter and decrease in number —progressively from the kinetochore distally—the ends are the last to be affected.

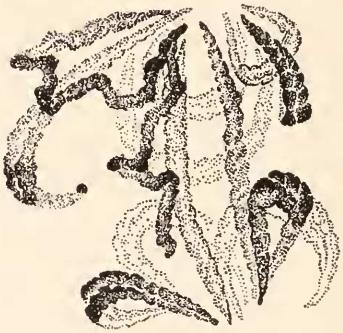
II. Kinetochore movements

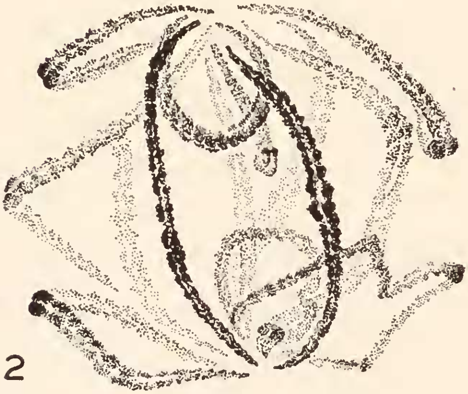
a. Kinetochore separation, spindle formation, and stretch.

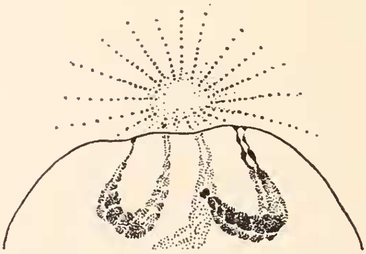
The nuclear membrane disappears early, while the nucleus is still in a late pachytene stage. The spindle forms immediately, filling the entire nuclear area, and undergoes a rapid elongation. Coincidently the bivalents are released from their polarized condition. Thereupon their ends appear flaccid and evince no further specific movement.

The two kinetochores of each bivalent now separate sharply and orient toward opposite poles (Fig. 5). This movement occurs while the bivalents are scattered throughout the nuclear area, their position reflecting the grouping of the preceding polarization (Figs. 5, 6, and 7). Bivalents lying close to one pole (upper right, Fig. 6) may show as extreme an initial separation of kinetochores as those in the equator.









(All drawings made with camera lucida at table level with Zeiss 2 mm., n.a. 1.3, obj. and 20 × oc.; enlarged with pantograph. Magnification as reproduced 2700 ×.)

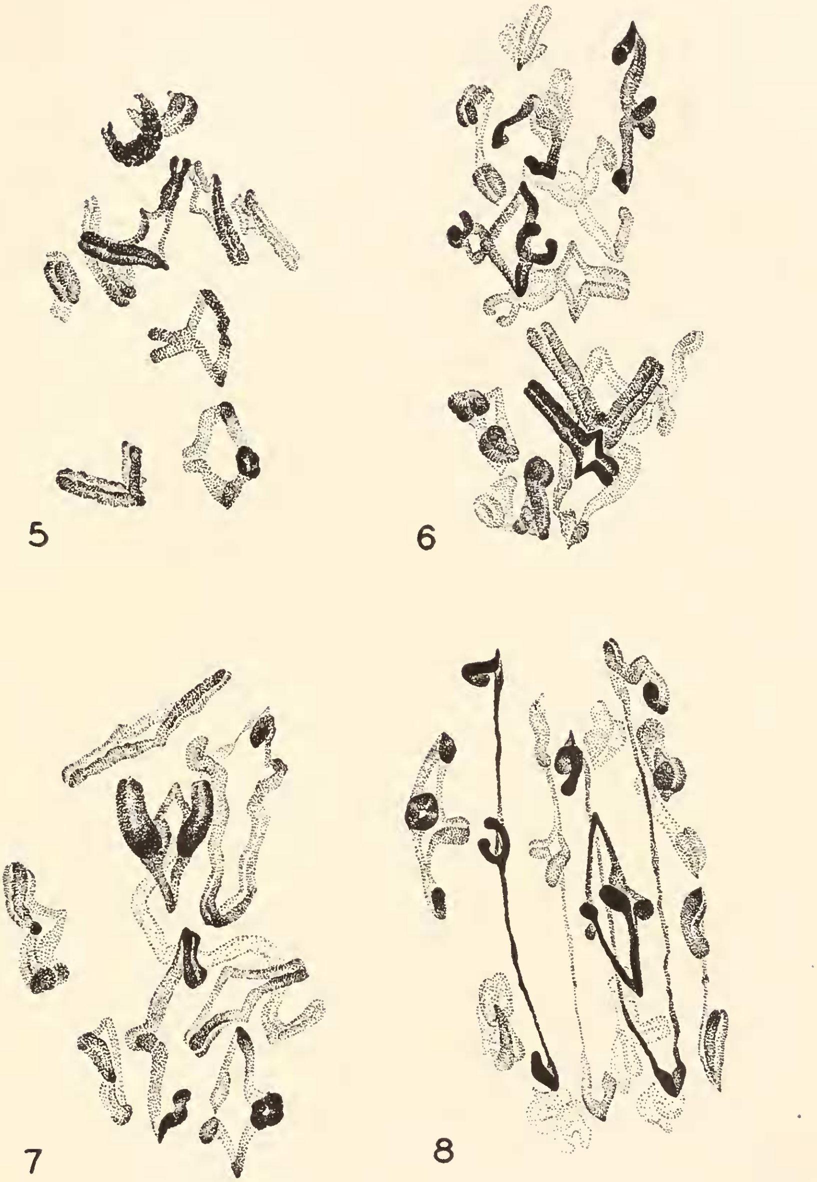
FIGURES 1 TO 4. *Stagmomantis carolina*, Virginia.

FIGURE 1. Non-polarized early pachytene; upper level only drawn; ends distributed at random. Feulgen.

FIGURE 2. Second polarization, in mid-pachytene. Feulgen; intact membrane and division centers at poles unstained.

FIGURE 3. Later stage in second polarization. Feulgen.

FIGURE 4. Detail of same after gentian violet and erythrosin; terminal chromomeres applied to membrane under center.



FIGURES 5 TO 8. *Stagmomantis carolina*, Virginia.

FIGURE 5. Early stretch; membrane gone, upper bivalents still polarized, other bivalents opening as kinetochores orient to poles. Feulgen.

FIGURES 6 AND 7. Early stretch; asynchrony of bivalents; secondary loci of separation between homologues; spindle elongation complete. Feulgen.

FIGURE 8. Mid-stretch; some bivalents not yet opened; open cross in several bivalents here and in Figure 9. Feulgen.

The events just described—break down of nuclear membrane, formation and elongation of spindle, separation and polar orientation of homologous kinetochores—take place with great rapidity as is shown by the rarity of these stages compared with those which precede and follow them. Other evidence supports this. Thus, cells in which the nuclear membrane has but just collapsed, as shown by the persistent polarization of some of the bivalents (top, Fig. 5), already show continuous spindle fibers between the centers, and a marked increase in inter-center distance over immediately preceding stages with membrane intact. Again, in stages such as Figure 6, whose closeness to Figure 5 is attested by evidence in the distribution of the chromosomes of their previous polarization, the spindle has already attained almost its maximum length. Indeed, the elongation of the spindle is always completed before all of the bivalents have been stretched open by the poleward movement of their kinetochores. [Measurements of spindle length at these and later stages are given in Table 1; a consideration of the role of spindle elongation in the kinetochore movement is reserved for the discussion—in comparison with data from the other species studied.]

The movement of homologous kinetochores toward opposite poles continues—often to an extreme degree. Not infrequently two thirds or more of the total spindle length may be traversed by the separating kinetochores of a given bivalent. In the process, the homologous chromosomes of each bivalent are stretched and pulled apart, retaining only terminal or subterminal connections in one or both arms (Figs. 8 and 9). The resulting attenuation of the stretched chromosomes is extreme, often appearing to approach the breaking point (note especially the middle bivalent of Figure 8). A pronounced asynchrony characterizes the stretching process among the different bivalents. In its early stages, as pointed out above, there seems no correlation between position in the spindle and time and degree of stretch (Fig. 6); later stages however show the most extreme stretch in bivalents equatorially placed on the spindle, while those nearer the poles are belated in opening (Figs. 8 and 9). Eventually all the bivalents are stretched open,—but the asynchrony is so great that the stretching process actually overlaps the recontraction of the bivalents which follows it.

Due to the shortness of the pairing segments in the sex chromosomes, their kinetochores are never so close together as those of the autosomal bivalents. Furthermore, the chromosomes of the sex trivalent, unlike those of autosomal bivalents, begin to separate and have already assumed an end to end alignment (X_1–Y–X_2) before the stretching process is initiated. (This point is difficult of demonstration in Stagmomantis but is clearly evident in Paratenodera and Choeradodis.) As the spindle forms in the nuclear area the kinetochores of the sex trivalent move toward the poles. Their movement, unlike that of the bivalents, is a random one,—not determined by repulsion between homologous kinetochores. Thus one X and the Y may move toward one pole and the other X toward the opposite pole; both Xs may go toward one and the Y toward the other pole; or, one X may pass toward each pole while the Y is stretched between them (Figs. 8, 9, and 10). Occasionally a trivalent shows all three kinetochores near one pole,—but it is impossible to distinguish these positively from trivalents which have not yet oriented. Apparently the direction of kinetochore movement is toward the nearer pole, determined by the chance position of the chain of three chromosomes at the time of the formation of the spindle.

b. Re-approach of homologous kinetochores.

The extreme stretching of the meiotic chromosomes is followed by their gradual re-contraction and the re-approach of their widely separated kinetochores. This movement seems to be brought about by the resumption of the normal coiling of late prophase, previously interrupted by the stretching process. A slight but consistent decrease in spindle length during the assumption of the compact form of final metaphase (measurements in Table I) undoubtedly expedites the process. The outline of the chromosomes becomes smoother and their staining capacity greater as contraction proceeds, suggesting the deposition of matrical material during this time. This feature, together with the degree to which the bivalent has been opened, permits one to distinguish between those bivalents in process of stretching and those undergoing the ensuing contraction. The precise time at which half spindle components form between the kinetochores and the centers is uncertain, due to the difficulty of differentiating them from the mass of fine fibrils making up the continuous spindle. However, at the period of maximum stretching many bivalents show unmistakable half spindle components. The re-contraction of the stretched chromosomes, and the re-approach of the homologous kinetochores, is thus effected despite any pull or resistance that may be offered by the half spindle components.

Table I

Kinetochore movement and spindle elongation

Measurements in ocular micrometer units; each figure is the average of some ten measurements.

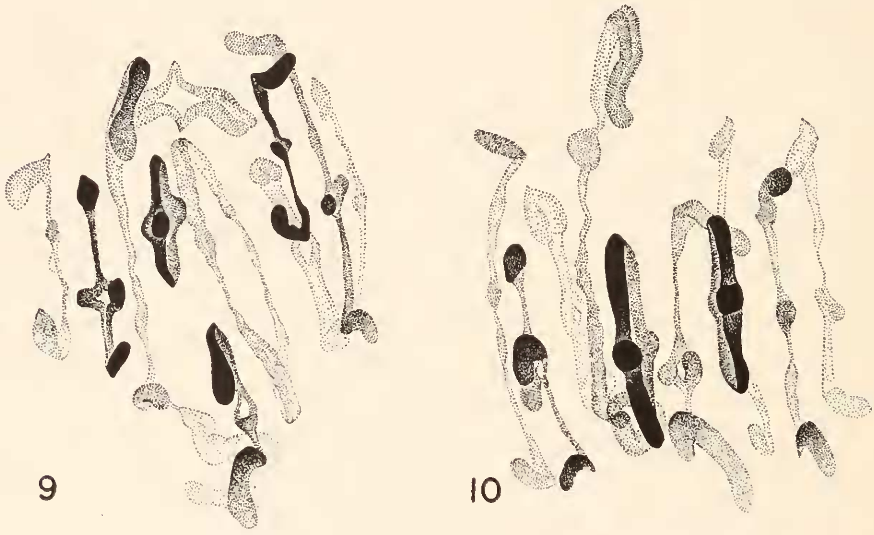
Species	Inter-center Distance				Spindle elongation during stretch	Inter-kinetochore Distance			Kinetochore separation during stretch	Kinetochore separation at metaphase
	Pre-stretch: membrane intact[1]	Early stretch: no membrane, no plate	Late stretch: plate forming	Compact metaphase: plate completed		Pre-stretch; membrane intact[1]; (av. max. sep.)	Maximum stretch; (in longest rod bivalent)	Compact metaphase; (in longest rod bivalent)		
Stagmomantis carolina, Va.	14.4	26.3	25.9	23.0	11.9	0.0	13.0	7.0	13.0	6.0
Stagmomantis carolina, B. C. Is.	19.0	24.2	24.1	22.7	5.2	2.0	10.5	7.2	8.5	3.3
Paratenodera sinensis	16.8	24.0	23.1	21.6	7.2	0.0	9.2	6.6	9.2	2.6
Liturgousa annulipes	11.5	24.5	23.9	24.0	13.0	2.1	10.6	9.2	8.5	1.3
Choeradodis rhombicollis	26.3	28.7	27.6	26.6	2.4	2.0	7.1	6.1	5.1	1.0

[1] Except in Liturgousa; here the inter-center distance recorded is the maximum attained before the orientation of the kinetochores to the centers but after the breakdown of the nuclear membrane.

The extent of these two opposite movements of the kinetochores,—first the violent movement of homologous kinetochores toward opposite poles, second their gradual re-approach to assume the position characteristic of metaphase,—is shown by measurements of the distance between the kinetochores of a given bivalent at the different stages. In the longest rod-shaped bivalent at the period of maximum stretch the distance between opposing kinetochores is 13 ocular micrometer units; at metaphase it is 7 units (each figure is the average of measurements in 10 nuclei). Since there occurs almost no separation of kinetochores prior to spindle formation in the Virginia Stagmomantis, the first figure gives the relative distance traveled by the kinetochores in their pre-metaphase poleward movement. In their re-approach the kinetochores retrace nearly half this distance. I would emphasize again the asynchrony of the different bivalents in these two movements; the stretching of laggard bivalents continues *pari passu* with the contraction of those which were first stretched open. Thus the kinetochores of one bivalent may be moving apart, toward opposite poles, while those of others are re-approaching each other.

c. Formation of metaphase plate

While the contraction of the bivalents and the re-approach of their kinetochores is under way, a new movement of the chromosomes is initiated. From a dispersed distribution through the whole spindle, the bivalents gradually shift into the equatorial region (Figs. 9 and 10). Each bivalent retains its bipolar orientation during this movement and moves as a whole toward the equator. The asynchrony noted in the two preceding movements is maintained in this also: movement toward the equator proceeds concomitantly with the re-contraction of the bivalents, and may even overlap the stretching of the most belated of them. The spacing of the chromosomes at the equator is at first wide and open—but as the maximum degree of contraction is reached they move in and form a fairly closely spaced metaphase plate. The accuracy of the



FIGURES 9 AND 10. *Stagmomantis carolina*, Virginia.

FIGURE 9. Late stretch; one bivalent still unopened; movement to equator started; X_1X_2Y in reorientation. Feulgen.

FIGURE 10. Early stage in metaphase orientation; X_1X_2Y mal-oriented. Feulgen.

seriation of stages in these movements of late prophase is attested by the fact that early anaphases always show the chromosomes in the compact form and closely spaced arrangement of the final metaphase.

Perhaps the most baffling feature of the metaphase orientation lies in the movements of the sex trivalent. We have seen that it assumes, at random, a variety of orientations during the stretching process. If the two Xs have moved to one pole and the Y to the other, the movement to the equator proceeds as in the bivalents simply by contraction of the chromosomes and a shifting of the whole configuration, with its orientation unaltered, into position in the metaphase plate. But if any other orientation be assumed during the stretch—if one X goes to each pole with the Y stretched between them, or if one X and Y pass to one pole while the other X goes to the opposite pole—a re-orientation ensues. One or more kinetochores shift their position and move through the spindle so as to bring the two Xs opposite to the Y on either side of the equator. Thus one kinetochore actually changes its orientation from one pole to the other and moves from a position close to one center to the opposite side of the equator —a maneuvre difficult indeed to visualize in terms of the mechanics of mitosis. Variation in the timing of the stretching of the trivalent and of its re-orientation, relative to the activities of the bivalents, makes it difficult to seriate its movements. But the trivalent shown in Figure 9 is probably undergoing re-orientation; the X near the upper pole is still under tension and maintains the orientation assumed in the stretch, while the lower X shows no tension and its kinetochore is in process of shifting toward the upper pole. What happens to the half spindle components during re-orientation would be of great interest, but I have not been able to follow it. Half spindle fibers are occasionally clearly visible during the stretch and are again well marked at metaphase; it is possible that they are lost and reform anew during the re-orientation.

Although the details of the process of re-orientation thus cannot be followed with certainty,—there can be no doubt that it actually takes place. Mal-orientation is encountered in some 50 per cent of the nuclei during the period of the stretch [56 cases were observed among 105 counted],—while at the final metaphase it is extremely rare [three cases in 105]. Clearly, therefore, the great majority of those trivalents mal-oriented during the stretch successfully re-orient by final metaphase.

III. Bivalent structure and chiasmata

Throughout pachytene the homologous chromosomes of each bivalent retain their close parallel association, with but a slight tendency to separate at the kinetochore region (Figs. 2 and 3). Diplotene and diakinetic stages as ordinarily recognized are absent: they are replaced by the forcible stretching open of the bivalents as the spindle forms, and the two kinetochores of each bivalent move toward opposite poles. As stretching proceeds it becomes evident that the kinetochore loop is not always the only locus of separation between homologues (note the two centrally placed bivalents in Figure 5). These openings, loops or half loops, alternate with persistently paired regions which resist the opening out process (Figs. 5 and 6). The paired segments may be terminal or interstitial, in one arm or in both; they vary in number from one to a maximum of three per bivalent. When first observable the openings between the paired regions appear to lie in the same plane, but as the tension in the kinetochore loop increases they may assume alternating planes (Fig. 6, middle left bivalent). The chromatids of each chromosome have not yet separated, and even the line of demarcation between chromosomes cannot be followed in the closely paired regions. It is thus impossible to determine whether or not chiasmata are present in the persistently paired regions. At the stage of maximum stretch, however, open cross formations are frequently encountered either at one end of a bivalent, giving the rod-shaped configuration, or at both with a resulting ring configuration (Fig. 8). In these bivalents it is clear that non-sister chromatids are associated distal to the opening of the cross.

No open cross configurations have been found in the sex trivalent. There is thus no evidence available as to whether or not the terminal adhesions of X_1, Y, and X_2 are of chiasmatal origin.

The final form of the bivalents at completed metaphase is fairly constant (Fig. 11). The most frequent complement comprises three rings and nine rods; rings vary in number from none to four per nucleus—rods show the corresponding range of 12 to eight. The open cross may persist to metaphase in from one to four bivalents, but the terminal connection is more commonly a swelling or lump, sometimes bipartite.

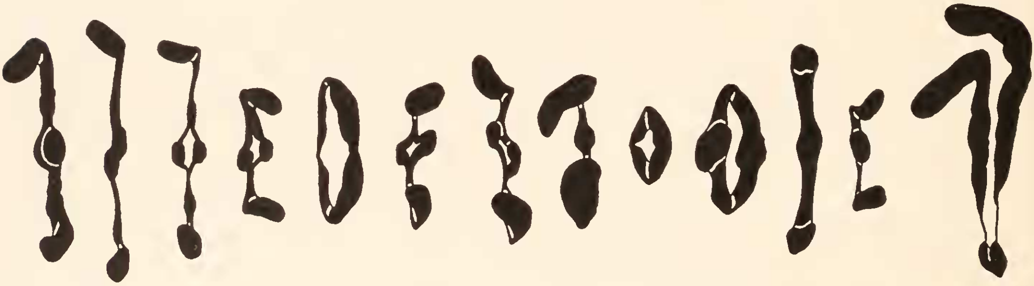
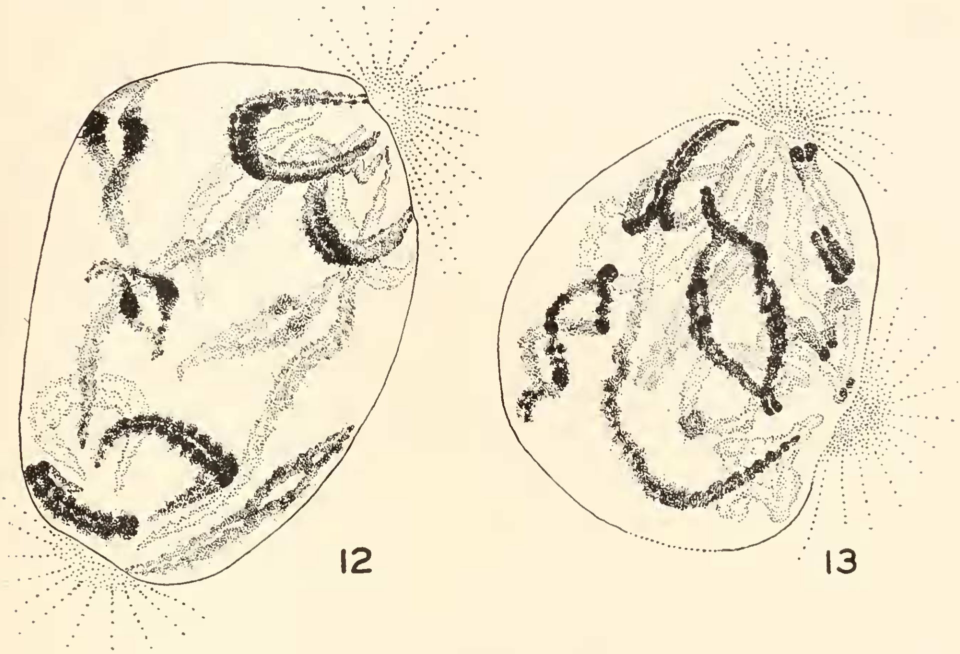


FIGURE 11. *Stagmomantis carolina*, Virginia. Bivalents and sex trivalent at metaphase. Gentian violet.

Stagmomantis carolina *from Barro Colorado Island*

Taxonomically indistinguishable, *Stagmomantis carolina* males from Barro Colorado Island and from Virginia are also identical in chromosome complement as observed in spermatogonial and meiotic metaphases. Striking and constant differences, however, characterize the meiotic prophase in the two types.

In the Barro Colorado material the pachytene polarization is initiated always at a later stage in the development of the bivalents than in the Virginian, and is of shorter duration (compare Figures 12 and 2). Moreover there is here no constant correlation between time of polarization, degree of separation of the centers, and the stage of bivalent development. Thus in Figure 13 the polarization centers are active while still relatively close together, but the bivalents are in a more advanced stage than those of Figure 12 in which the centers are already at opposite sides of the nucleus. The time of breakdown of the nuclear membrane also varies relative to the degree of separation of the centers. When the centers separate early their passage to opposite sides of the nucleus is accompanied by a marked elongation of the whole nucleus (Figure 12 is typical) along the inter-center axis. Thus when the spindle forms in the nuclear area on the collapse of the membrane, the average inter-center distance is already considerably greater than in the Virginia type. Some elongation of the spindle follows immediately on its formation but the total length achieved is somewhat less and the average amount of elongation considerably less than in the Virginia material (measurements in Table I). The maximum separation of homologous kinetochores during the stretching process is definitely less in the Barro Colorado type; this is probably dependent both on the more advanced stage of bivalent contraction and the lesser spindle elongation.



FIGURES 12 AND 13. *Stagmomantis carolina*, Barro Colorado.

FIGURE 12. Second polarization, late pachytene; early separation of homologous kinetochores. Haematoxylin.

FIGURE 13. Second polarization with centers till close together; early breakdown of membrane; advanced stage of bivalent opening. Haematoxylin.

A highly significant feature of the Barro Colorado type lies in the timing of the separation of homologous kinetochores. In the Virginia form this occurs simultaneously with the orientation of the two kinetochores of each bivalent to opposite centers—and only after membrane collapse and spindle formation. In bivalents of the Barro Colorado material, the homologous chromosomes show a marked localized separation at the region of the kinetochores while the nuclear membrane is still intact (Fig. 12). By the time the membrane gives way the separation of homologues, initially localized at the kinetochore region, has spread distally until in some cases the bivalent appears as a ring with only the terminal regions of the chromosomes still parallelly associated (Fig. 13). It is clearly evident in these stages (Figs. 12 and 13), that the plane of separation between homologous kinetochores bears no relation to the future spindle axis. This fact, together with the persistance of the nuclear membrane during the initial separation, demonstrates therefore that this first phase in the kinetochore movement is independent of centers and spindle.

With the collapse of the membrane and formation of the spindle in the nuclear area, the already widely separated kinetochores of each bivalent orient and move toward opposite poles (Fig. 14). As in the Virginia type, this first phase in the stretching process occurs while the chromosomes are scattered through the whole spindle; Figure 15 is a particularly striking example with four bivalents, all placed well above the equator, showing the movement of the kinetochores to opposite poles. In this and succeeding stages however the stretch is always most extreme in equatorially placed bivalents.

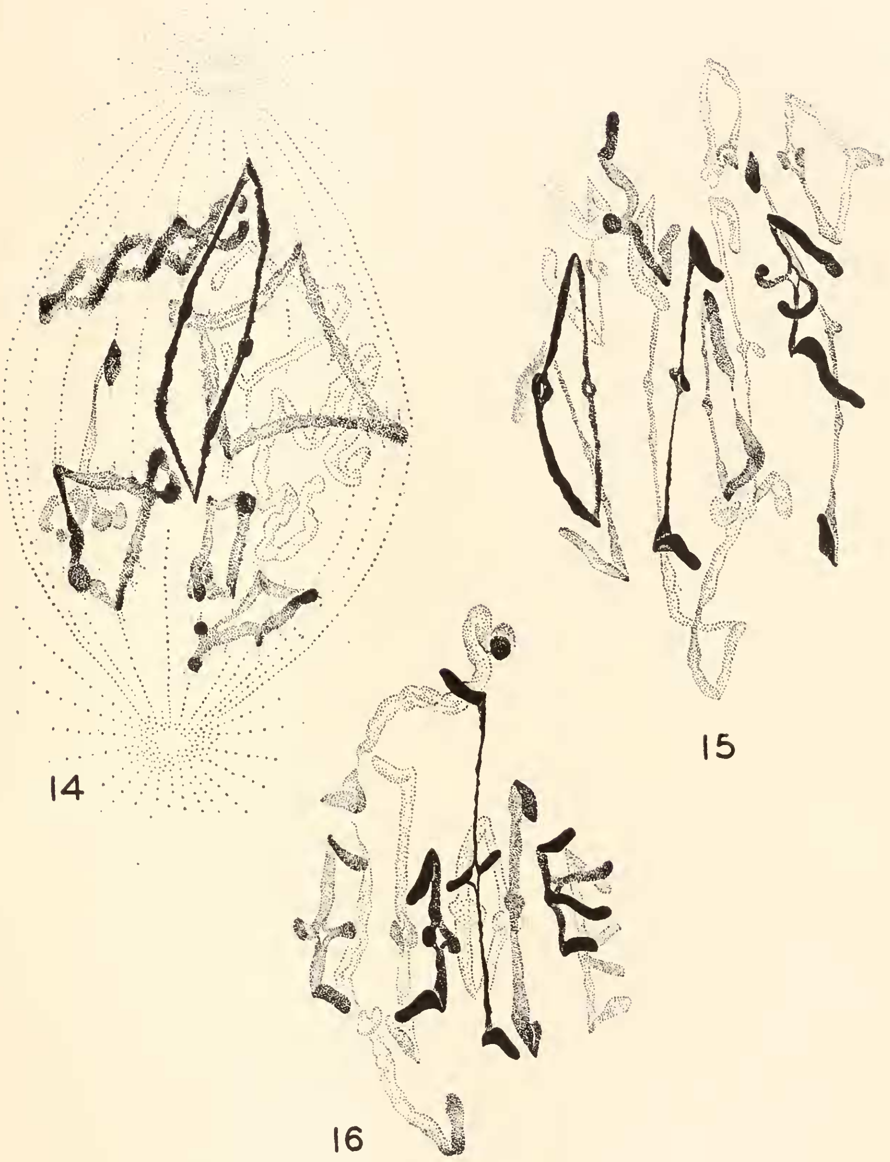
The asynchrony of the bivalents in the stretching process is equally marked in both types (compare Figures 14 to 16 with 6 to 9). In both the maximum spindle length is attained before all of the bivalents have completed the stretching process. The structure of the bivalents as disclosed during the stretch is also identical in the two types. Re-contraction of the chromosomes, re-approach of homologous kinetochores, and the movement to the equatorial plate proceed similarly. The basic difference between the two types thus lies in the timing of spindle formation relative to the stage of bivalent development. The more precocious spindle formation in the Virginia form superimposes the initial separation of homologous kinetochores and their bipolar orientation. With the delay in spindle formation in the Barro Colorado type the two processes are seen to be distinct; the initial separation of kinetochores is not determined by the centers nor the developing spindle.

Paratenodera sinensis

The chromosomes of this species have been studied and figured by King (1931) and White (1941). The diploid number of the male is 27, 12 pairs of autosomes plus X_1, X_2 and Y.

I. Second polarization

Pachytene polarization in Paratenodera presents an interesting and significant variation from the pattern observed in Stagmomantis. Usually polarization is not marked until the bivalents have condensed into short thick rods. No diplotene opening out, however, accompanies this prolonged period of contraction. The bivalents, scattered widely in the nucleus, evince some tendency toward peripheral distribution; some loose collocation of ends may persist from the leptotene zygotene bouquet, but no regular orientation is apparent. Asters form while the centers are still fairly close together. Only then do the chromosomes become definitely polarized, forming two loose aggregations close to the nuclear membrane and underlying the centers (Fig. 18). So compact are the bivalents at this stage that it is impossible to say whether their ends only or the whole mass is involved in the polarization. However the time at which the

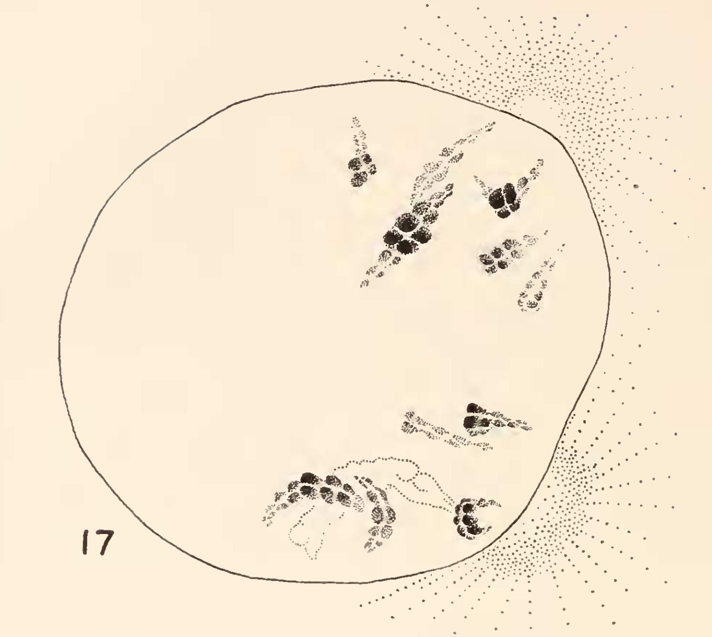


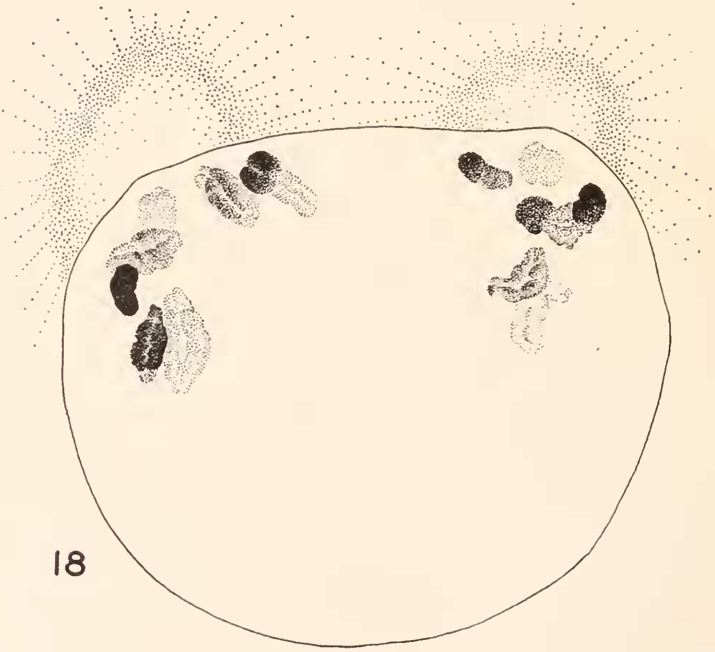
FIGURES 14 TO 16. *Stagmomantis carolina*, Barro Colorado.

FIGURE 14. Early stretch. Haematoxylin.

FIGURE 15. Mid stretch; bivalents still scattered; early stage in open cross in one bivalent. Gentian violet.

FIGURE 16. Early stage in metaphase orientation; mal-orientation of sex trivalent. Gentian violet.





FIGURES 17 AND 18. *Paratenodera sinensis.*

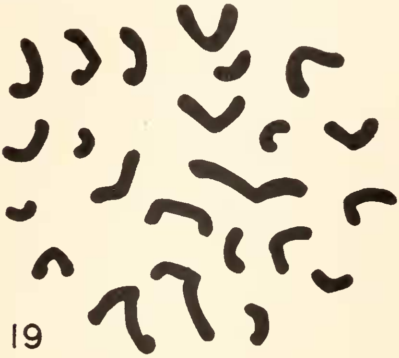
FIGURE 17. Early second polarization with chromosome ends polarized; sex trivalent in outline. Gentian violet.

FIGURE 18. Typical second polarization. Haematoxylin.

centers become active varies in relation to the stage of chromosome contraction. In a small percentage of the cells the asters form while the bivalents are still fairly long, and it is then evident that the ends of the chromosomes are specifically involved (Figure 17) just as in Stagmomantis. The Paratenodera material thus affords a series of transitional phases linking bouquet formation with the type of late prophase polarization found in Anisolabis (Schrader, 1941a) in which a specific activity of chromosome ends is not evident.

II and III. Kinetochore movements and bivalent structure

Events subsequent to the cessation of pachytene polarization follow the same general course as in Stagmomantis. Like the Virginia type of the latter, the bivalents show no marked separation of kinetochores prior to the collapse of the membrane. Formation and elongation of the spindle coincides with the initial opening out of the bivalents but the stretching process continues after spindle growth is complete. Degree of spindle elongation and intensity of stretch are intermediate between the Virginia and Barro Colorado types of Stagmomantis (measurements in Table I). Re-contraction of bivalents, re-approach of kinetochores, and movement to the equator show no significant differences from the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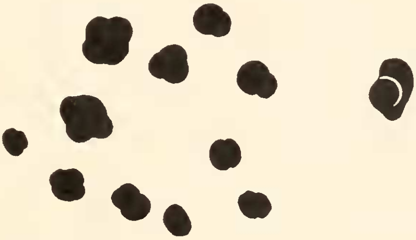
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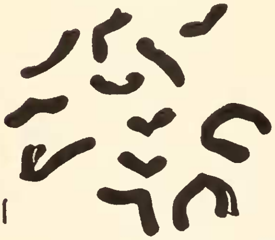
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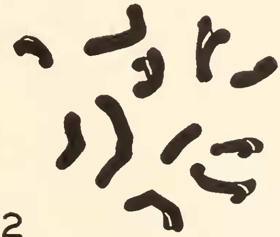
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FIGURES 19 TO 22. *Liturgousa annulipes.*

FIGURE 19. Spermatogonial metaphase. Gentian violet.
FIGURE 20. First meiotic metaphase. Univalent X off plate at right, viewed from open end of V. Gentian violet.
FIGURE 21. Second meiotic metaphase—11 autosomes and X. Gentian violet.
FIGURE 22. Second meiotic metaphase—11 autosomes and no X. Gentian violet.

conditions in Stagmomantis. The structure of the bivalents also corresponds closely in the two genera.

Liturgousa annulipes

The chromosomes of this species have not previously been recorded. The diploid number of the male as seen in spermatogonial metaphase is 23, comprising 11 pairs of autosomes and a single X (Fig. 19). The kinetochore is approximately median in six pairs of autosomes, subterminal in five, and submedian in the X (Figs. 19 and 27). The X passes undivided to one pole at the first division and divides at the second. Second metaphase shows the expected chromosome sets of 12 and 11 (Figs. 21 and 22).

I. Second polarization

The pachytene polarization of the bivalents is very slight (Fig. 23). Many cells show none at all, and the maximum observed involves the orientation of one or both ends of at most three to five bivalents—probably those which happened to lie near the center when it became active. The time of polarization coincides, as in all the other species studied, with the first formation of astral rays. In Liturgousa, however, the latter make their appearance while the centers are still either undivided or so close together that only a single center of polarization is produced.

II. Kinetochore movements

a. Kinetochore separation, spindle formation and stretch.

As in the Barro Colorado Stagmomantis, the initial separation of homologous kinetochores is independent of division centers and spindle. This is shown, as in the former case, by the marked "repulsion" of kinetochores which occurs, in many bivalents, from mid-pachytene on (Figs. 23 and 24). Since the nuclear membrane is still intact, the future spindle axis not yet established, and the plane of kinetochore separation is random, any influence of the achromatic figure on the kinetochore movement is excluded.

The nuclear membrane breaks down while the centers are still close together (Fig. 25). The subsequent reaction of kinetochores to centers is slow in comparison with the other species, but gradually all the bivalents become oriented with one kinetochore moving toward each pole. Meanwhile the spindle forms and elongates. Due to the late separation of the centers spindle elongation after membrane collapse is greater than in any of the other species (Table I). But, as in the other forms, its maximum length is attained before the stretching of the bivalents is completed. A late stage in the stretching process, with one bivalent not yet completely opened, is shown in Figure 26.

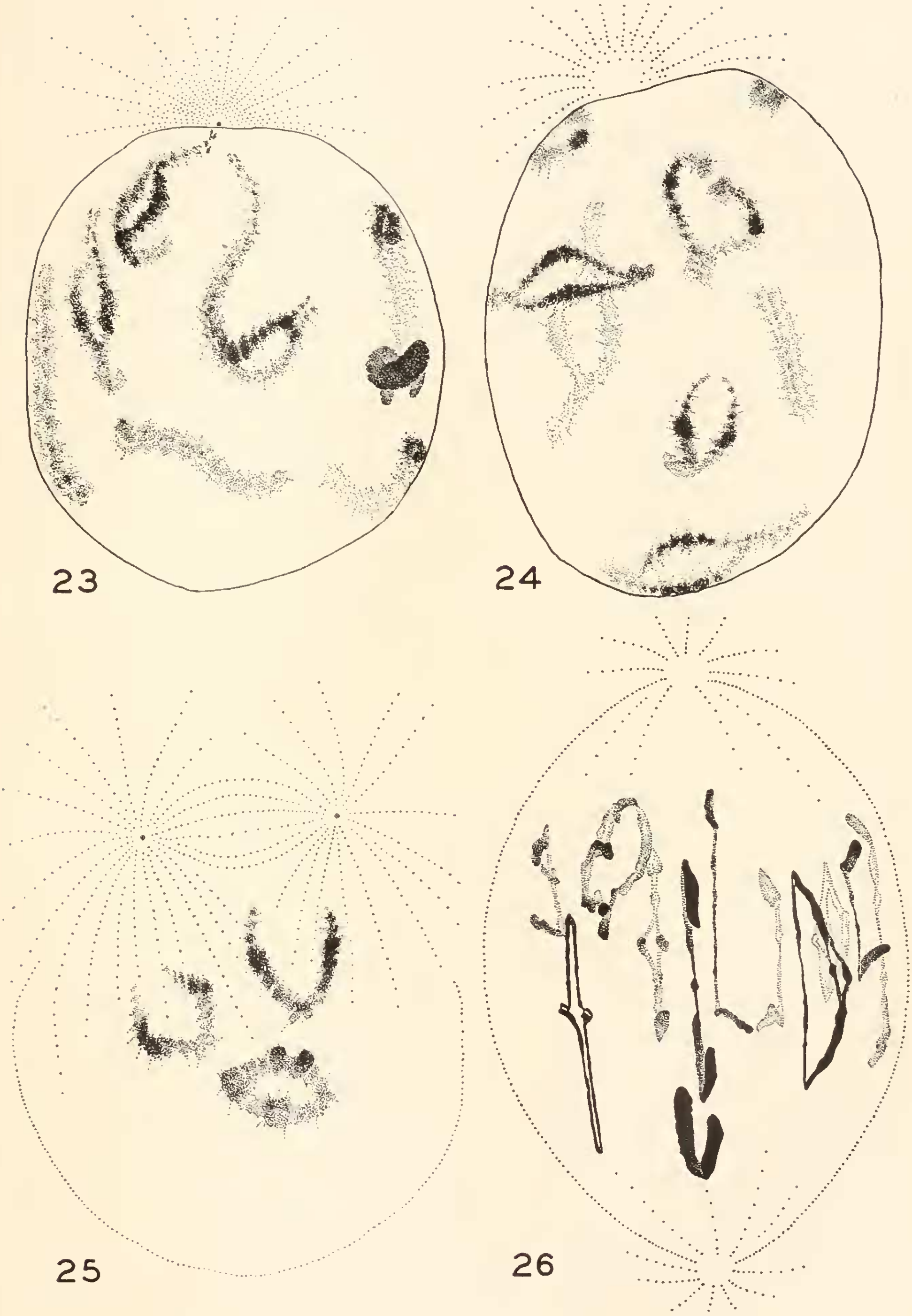
FIGURES 23 TO 26. *Liturgousa annulipes.*

FIGURE 23. Second polarization; slight orientation of chromosome ends near center; division centers not yet separated. Haematoxylin.

FIGURE 24. Same stage—no polarization; kinetochores separating in various planes. Haematoxylin.

FIGURE 25. Slightly later; membrane gone; centers separating; delayed orientation of kinetochores. Haematoxylin.

FIGURE 26. Late stretch; metaphase orientation started. Gentian violet.



FIGURES 23 TO 26.

b. Re-approach of homologous kinetochores; metaphase plate formation.

The re-contraction of the bivalents, re-approach of the homologous kinetochores, and the gradual movement toward the equatorial region proceed precisely as in Stagmomantis.

III. Bivalent structure and chiasmata

In spite of the markedly early separation of the kinetochores and the consequent opening out of the bivalents before spindle formation, the chromatid structure of the bivalents is not analyzable at this stage. The separation of homologous chromosomes, at first localized in the kinetochore region, continues until a large kinetochore loop is formed, or, in some bivalents, the homologues become completely disjoined except for one short paired segment. Sister chromatids remain closely associated and cannot be traced through the persistently paired regions. That the separation of homologous chromosomes is not solely due to kinetochore movement is shown by the presence in some bivalents of a second locus of opening out in addition to the kinetochore loop or half loop. This is apparent in the separation of the ends of the horizontally placed bivalent at the mid left in Figure 24, and in all bivalents of Figure 25. After spindle formation and during the stretching process the open cross formation is encountered in several bivalents (Figures 26 and 28, a and b). The frequency of open crosses is 11.6 per cent (in 249 counted) during the stretch, and is reduced to 5.4 per cent (in 351) by final metaphase.

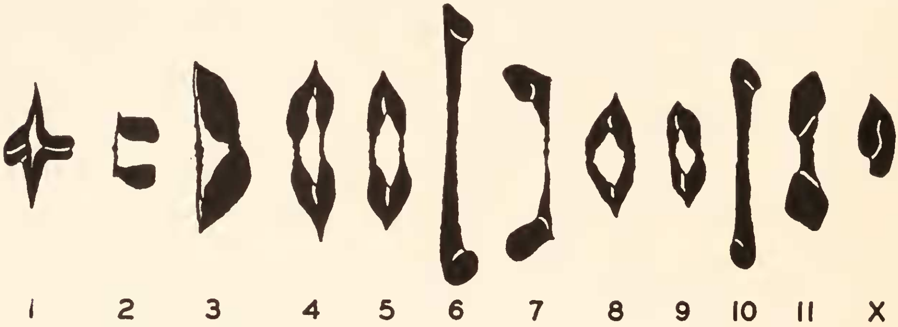


FIGURE 27. *Liturgousa annulipes.* Eleven bivalents and X at completed metaphase. Gentian violet.

The open cross configuration is usually accepted as evidence of a chiasma in process of resolution by the rotation of the arms of the bivalent. We might then assume, as White (1941) has suggested, that the terminal connections between the homologous chromosomes of the bivalents at metaphase are of chiasmatal origin. If a chiasma were present in each arm, two terminal connections would be formed—giving a ring bivalent at metaphase; if only one arm contained a chiasma, a single terminal connection and a rod bivalent would result. The behavior of two of the Liturgousa bivalents does indeed support such an argument. Easily identifiable at metaphase is the small bivalent number 2 of Figure 27 in which a terminal connection between the short arms always persists throughout the stretch, while the long arms are always free. The large bivalent number 3 of Figure 27 has arms of similar proportions, but in its case the long arms as well as the short ones are invariably connected. Moreover, in this bivalent the open cross formation may occasionally be observed in the long arms during the stretch (Fig. 28, c). Since the short arms only are subjected to stress in both bivalents, it seems reasonable to assume that the difference in behavior of the long arms is associated with the presence or absence of chiasmata. On this assumption, if no chiasma is present, the homologues separate completely and early as in the long arm of number 2, while the presence of a chiasma results in a persistent terminal connection as in the long arm of number 3.

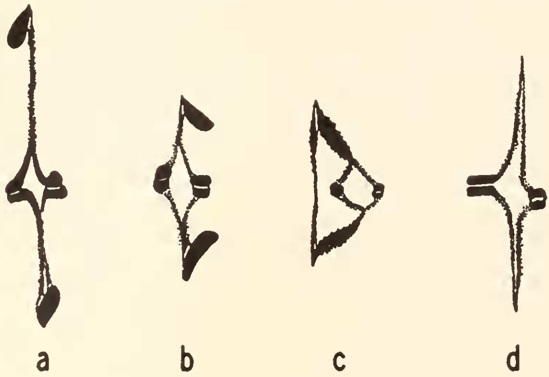
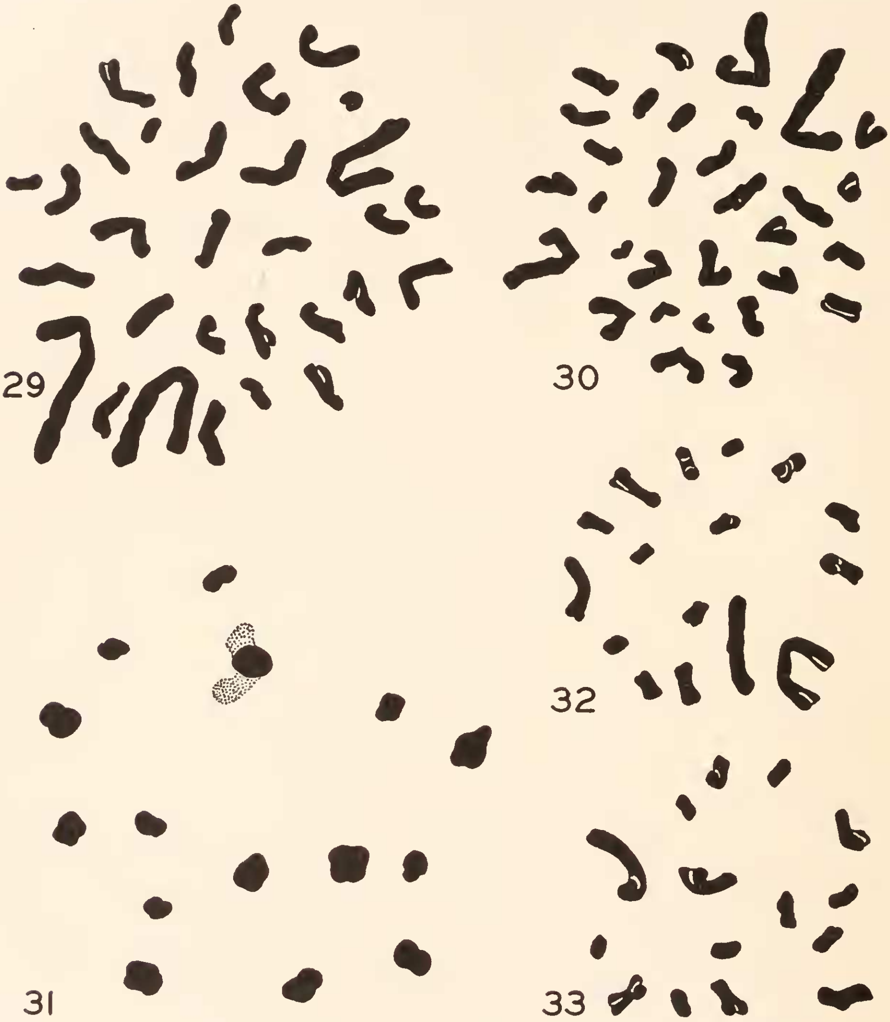


FIGURE 28. *Liturgousa annulipes.* a and b, open cross formation in rod bivalents; c, open cross in bivalent no. 3; d, bivalent no. 1 during stretch. Gentian violet.

It does not follow, however, that chiasmata are the sole and essential factor in maintaining the association of homologues. One is not justified in generalizing from the behavior of these two bivalents. Some bivalents never show the open cross or any other evidence of chiasmata; their homologues separate during the stretch with sister chromatids persistently associated; the connection between chromosome ends in these cases carries no necessary implication of previous chiasmata. Even stronger evidence is afforded by the behavior of bivalent number 1 (Figure 27). It is readily identifiable at metaphase because, alone of all the bivalents, its constituent chromosomes are in parallel association except at the kinetochore. It thus, except for a somewhat closer association of chromatids, strikingly resembles the bivalents of Callimantis (White, 1941; Hughes-Schrader, 1943) in which the absence of chiasmata in late prophase and metaphase is definitely demonstrable. But unlike Callimantis, in Liturgousa this bivalent is subjected to the same stretching process which reveals in the other bivalents of the set those open crosses and terminal connections suggestive of chiasmata. It is, therefore, evidence of a real difference in the factors determining bivalent structure to find that the homologous chromosomes of bivalent 1 separate smoothly and progressively during the stretch, disclosing no evidence of chiasmata, and making no terminal knots or adhesions. The paired region at the end of each arm decreases steadily in extent as the bivalent is stretched, but retains some parallel orientation of its chromosomes as long as it can be followed (Fig. 28, d). Re-contraction of bivalent 1 after the stretch brings the separated chromosomes back into parallel association throughout their length, except at the kinetochore. If the absence of chiasmata permits the chromosomes of the long arm of bivalent 2 to separate early and completely, why do the arms of number 1, apparently equally devoid of chiasmata, retain the parallel association of their homologues? Conversely, the pairing force operative in number 1



FIGURES 29 TO 33. *Choeradodis rhombicollis.*

FIGURE 29. Typical spermatogonial metaphase. Gentian violet and erythrosin.

FIGURE 30. Same, with maximum association of homologues. Gentian violet and erythrosin.

FIGURE 31. First meiotic metaphase; sex trivalent at upper center with Y at top focus and 2 Xs, stippled, at lower focus. Gentian violet.

FIGURE 32. Second meiotic metaphase, 14 autosomes plus 2 X. Gentian violet.

FIGURE 33. Same, with 14 autosomes plus Y. Gentian violet.

would seem to be absent in the long arm of number 2. Variation in the factors determining both the association of homologous chromosomes and the form of the metaphase configurations is thus clearly indicated for different bivalents within a single complement. Further consideration of these data is given in the discussion.

Choeradodis rhombicollis

The diploid number in the male of this species, as determined in spermatogonia, is 31—the highest number thus far found among mantids (Figs. 29 and 30). Williams' (1938) count of 27 is based on a single adult fixed without dissection, and his figures indicate that the material was inadequate to establish chromosome number and behavior.

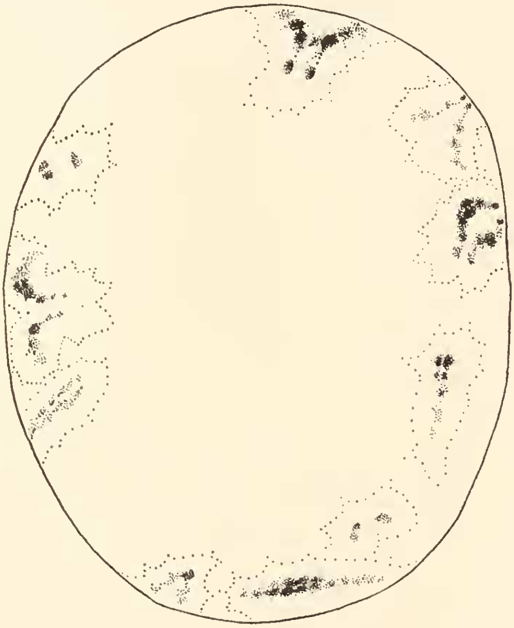


FIGURE 34. *Choeradodis rhombicollis.* Pseudo-diakinesis following diffuse late pachytene. No polarization; bivalents peripheral; erythrosin stained coagulum about chromosomes. Gentian violet and erythrosin.

The chromosome complement embraces 14 pairs of autosomes, four with median, 10 with subterminal kinetochores, and 3 sex chromosomes, X_1 X_2 and Y, with submedian kinetochores. Homologous chromosomes tend to lie near one another at spermatogonial metaphase; Figure 30 shows the maximum association observed, Figure 29 the more typical condition. The sex chromosomes are positively heteropycnotic in spermatogonial prophase and telophase, and lag in the anaphase movement.

Fourteen bivalents and a sex trivalent are formed at meiosis (Fig. 31). In general structure and behavior the trivalent corresponds with those described for other species. The proportions of the arms and the pairing relations at metaphase are shown in Figure 38. At the first meiotic division the 2 Xs pass to one pole, the Y to the other; the second metaphase accordingly shows either 15 or 16 elements (Figs. 32 and 33).

I. Second polarization

Leptotene, zygotene, and early pachytene stages correspond closely to those of the other species studied. In late pachytene however, the chromosomes become diffuse in outline, are almost unstainable in gentian violet and haematoxylin, and give but a faint Feulgen reaction. When later they again become stainable, the bivalents, already greatly shortened compared to the long threads of early pachytene, are found peripherally distributed close to the nuclear membrane (Fig. 34). Asters form about the centers, and these separate quickly to opposite sides of the nucleus, which may elongate slightly along the inter-center axis (Fig. 35); but the polarization is always slight and many do not reach the polar regions. So late is the polarization relative to the stage of contraction of the chromosomes that the latter appear to move as wholes. In a few nuclei, however, polarization occurs while the chromosomes are still relatively long, and then, as in Paratenodera, the ends alone orient to the center.

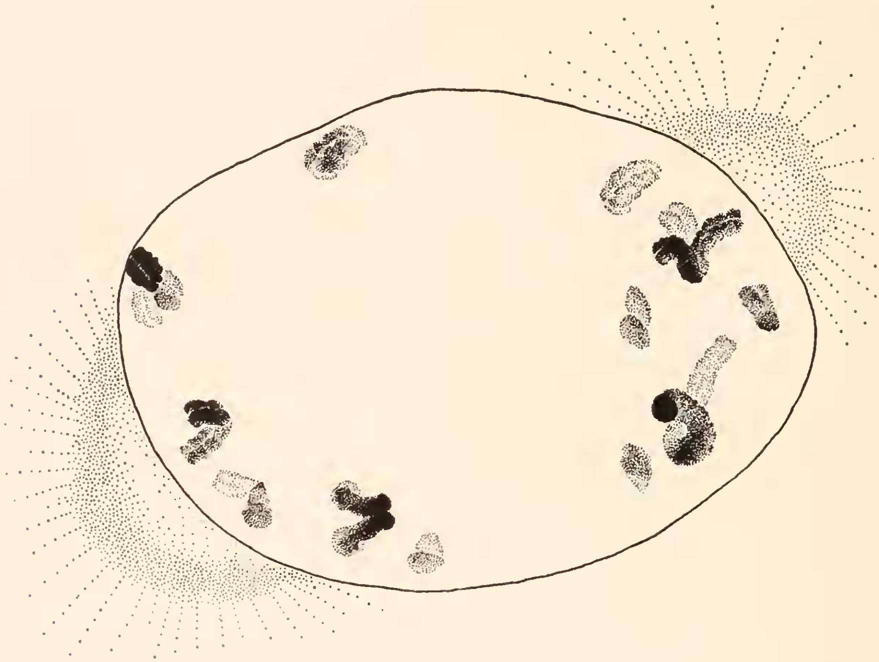


FIGURE 35. *Choeradodis rhombicollis.* Second polarization; chromosomes loosely aggregated near division centers. Haematoxylin.

II. Kinetochore movements

On the breakdown of the nuclear membrane the spindle forms in the nuclear area and quickly elongates. Due to the large size of the nucleus and the consequent wide separation of the centers, the length of the spindle is considerably greater than in any of the other species,—but the extent of its elongation is relatively slight (comparative measurements in Table I). As in the other species, spindle elongation is completed before the opening out of many of the bivalents. Indeed the orientation and movement of homologous kinetochores toward opposite poles takes place very gradually and with pronounced asynchrony in this species. Figure 36 is typical; the spindle has completed its elongation; of the bivalents some still retain the parallel association of their homologues except at the kinetochores, while others have completed the stretching process. The degree of stretching is very slight compared to the other species; the maximum separation of kinetochores observed is less than a third of the length of the spindle. The contraction of the bivalents to their final metaphase form is similarly slight. The further movements of the chromosomes to the equator and their orientation in the metaphase plate proceed as in the other species. The behavior of the sex trivalent parallels in essentials that of Stagmomantis. The early terminal alignment of the sex chromosomes, prior to the breakdown of the nuclear membrane, is especially clear in Choeradodis.

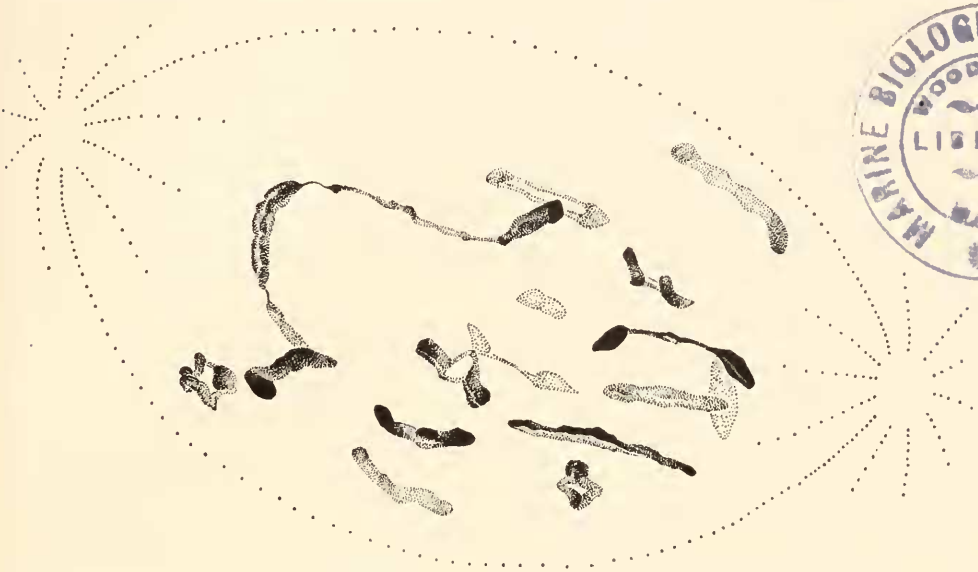


FIGURE 36. *Choeradodis rhombicollis.* Stretch: elongation of spindle completed; asynchrony of bivalents in opening out. Gentian violet.

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III. Structure of bivalents

Unfortunately in Choeradodis, in which among all the species thus far studied the closest approach to a regular diplotene-diakinetic opening out occurs, the structure of the bivalents is least analysable,—due to their small size and the diffuse condition of the chromatin in the critical stages. Certain significant features can, however, be established.

Already on emergence from the confused stage of late pachytene the bivalents show a marked degree of separation between homologues (Fig. 34). This may be uniform along their entire length, or variously accentuated in different regions. If uniform, the homologues are clearly separated and indubitably devoid of chiasmata (Fig. 37, a, after Feulgen, and e, gentian violet). [Occasionally a cross section of such a bivalent shows all four chromatids equally spaced (Fig. 37, f), but such figures are rare and I have not observed chromatid separation in any others.] Prometaphase bivalents of this type probably assume a Callimantis-like form at metaphase as in bivalent 2 in Figure 38, or if more widely opened, produce simple rings such as bivalents 1 and 3 in the same figure.

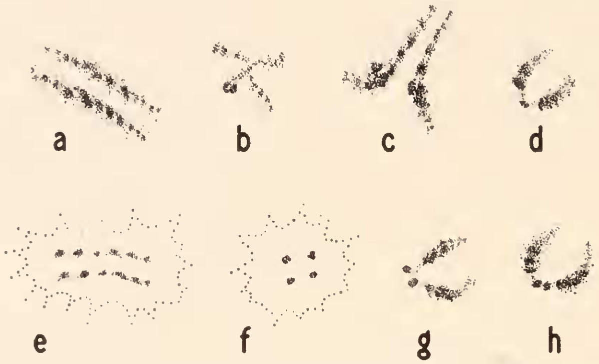


FIGURE 37. *Choeradodis rhombicollis.* Individual bivalents at pseudo-diakinesis. a–d, Feulgen; e–h, gentian violet.

In other bivalents, homologues are moderately and uniformly separated at one end, but flare widely apart at the other as though mutually repelling each other (Fig. 37, c). If at metaphase a terminal attraction operates between the paired ends when the poleward movement of the kinetochores has brought them into apposition, a rod-shaped bivalent, with no implication of previous chiasma, will result.

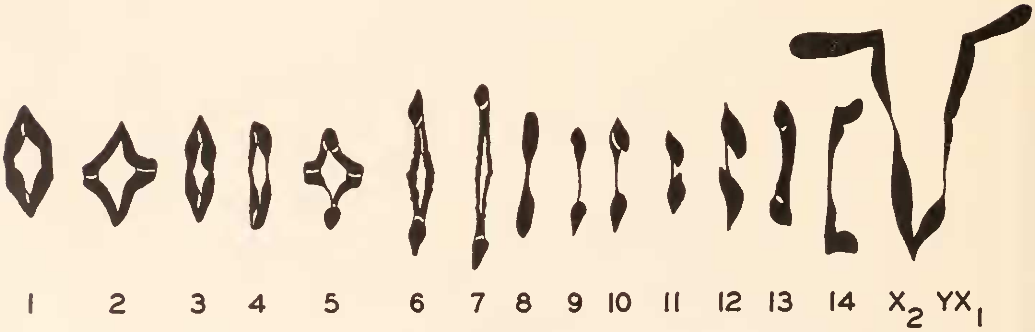
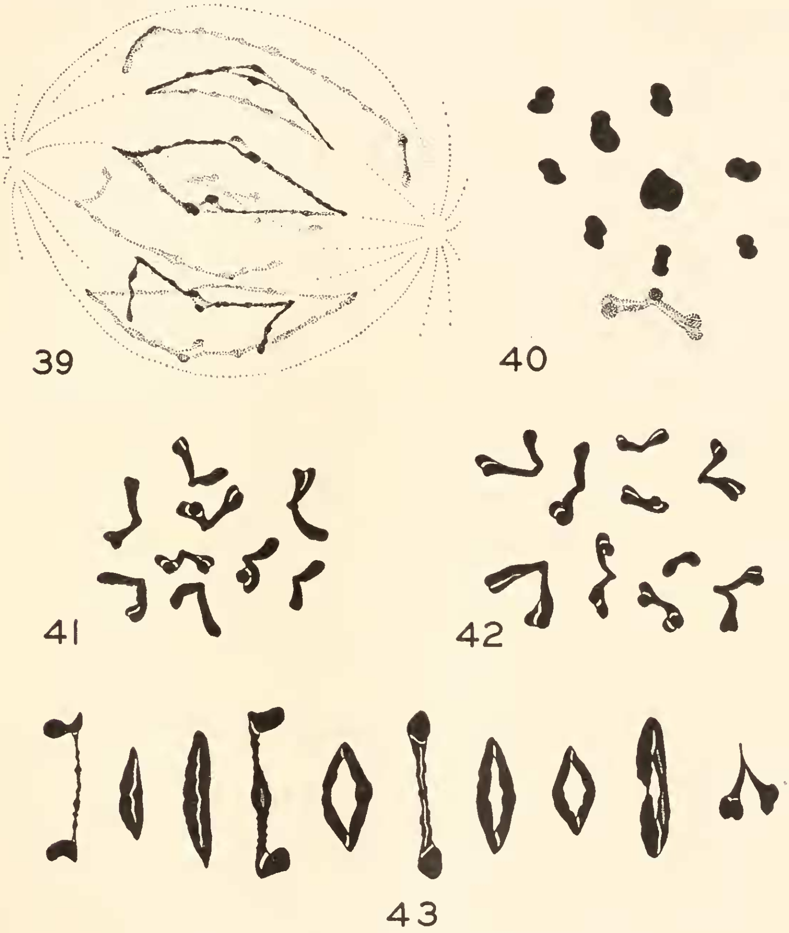


FIGURE 38. *Choeradodis rhombicollis.* Bivalents and sex trivalent at first metaphase. Gentian violet.

A third type of association is shown in Figure 37, b, d, g, and h. Here the persistently paired region, usually terminal or subterminal, is very short and the homologues separate widely throughout the rest of their length, with maximum separation usually at the kinetochore region. Homologous chromosomes are in contact in the paired region, but since chromatids cannot be distinguished it is impossible to tell whether or not a chiasma is present. The possibility that chiasmata are involved in these contact points is strengthened by the occasional occurrence of open cross formations in the later stages (Fig. no. 5). The frequency of the open cross is low,—slightly less than 1 per cent at metaphase.



FIGURES 39 TO 43. *Angela guianensis.*

FIGURE 39. Stretch stage. Haematoxylin.
FIGURE 40. First meiotic metaphase; X lies off plate at low focus. Gentian violet.
FIGURES 41 AND 42. Second meiotic metaphases. Gentian violet.
FIGURE 43. Bivalents and X at compact metaphase. Gentian violet.

Taken as a whole the Choeradodis data support the conclusion derived from the Liturgousa bivalents: the factors determining the association of homologous chromosomes in late prophase and the form of the bivalents at metaphase vary in different bivalents of a single species. Additionally Choeradodis demonstrates that in certain cases a pairing force independent of chiasmata is variously localized along the bivalent.

Angela guianensis

My material of this species is limited to a single adult male, in which the stages of spermatogenesis are incompletely represented. Its chromosomes have not previously been described. The diploid number in the male is 19, determined from meiotic metaphases (Figs. 40 to 43). Six pairs of autosomes and the univalent X have median—the others submedian kinetochores. At metaphase the bivalents assume the familiar rod or ring form; the number of rods varies from 3 to 5, of rings from 4 to 7; the most common complement being 3 rods and 6 rings (Fig. 43).

Early prophase is typical. Pachytene polarization is represented by but few nuclei but these indicate that it occurs relatively early as in the Virginia Stagmomantis. The most striking feature of the prophase is the extreme delicacy of the chromosomes at the time of stretching—a chromomeric structure is still present during the period of the stretch (Figure 39). Spindle formation seems to be more precocious than in any other species. The spindle is very compact, nearly spherical in form, and the stretched chromosomes are curved in conformity with it. During the stretching and subsequent re-contraction of the bivalents, open cross configurations are frequent,—nearly every nucleus in these stages showing two or three. These are resolved and are succeeded by terminal connections in final metaphase.

The low chromosome number, precocity of spindle formation, and frequency of open cross formation suggest that this species would reward further study.

DISCUSSION

I. Polarization

The second polarization of chromosomes in the mantid prophase is of especial significance in the analysis of the bouquet stage—so characteristic a feature of meiosis in many animals, and to a lesser extent, plant species. The interpolation of a non-polarized stage just prior to the second bouquet demonstrates that the latter cannot be interpreted simply as a passive relic of previous telophase orientation, but involves an active orientation of chromosomes. Furthermore, the second bouquet occurs at a stage more open to analysis than is the leptotene. It is thus possible to demonstrate the participation of three structural elements of the cell in the process of polarization: (1) the orientation movements are performed by the ends of the chromosomes; (2) the division center determines the focus of aggregation; and (3) the nuclear membrane is involved in polarization.

Evidence on all these points has long been available. Recognition has been delayed due to a confusion of two distinct types of orientation: (1) the active orientation of chromosomes to membrane and center so frequently encountered in meiotic prophase, and (2) the superficially similar Rabl orientation—a passive relic of previous telophase orientation—which has been demonstrated with perhaps equal frequency in mitotic prophase. Confusion has arisen because in meiotic division the first process is sometimes superimposed upon the second. If the true leptotene bouquet is formed in cells which retain the pre-meiotic telophase orientation of their chromosomes it may well be impossible to discriminate between two distinct but simultaneously acting factors—the Rabl or relic orientation and the active orientation of bouquet formation. A third factor may further complicate polarization; it lies in the mutual attraction, at certain phases in the mitotic cycle, of heteropycnotic regions of the chromosomes. If heteropycnosis is terminally localized in the chromosomes and the attraction operates simultaneously with peripheral distribution and a polarizing activity of the center, a bouquet formation will result (as, for example in Phrynotettix (Wenrich, 1916), Stauroderus (Corey, 1933, 1938), and several species of Edessa (Schrader, 1941b)). The numerous well established cases in which bouquets form without heteropycnosis, as well as those in which heteropycnotic regions aggregate without bouquet formation, show that two separate processes are involved.

The recent revival of the idea that the bouquet stage is conditioned by previous telophasic orientation (Atwood, 1937; Hiraoka, 1941; Smith, 1942) demands reconsideration of the evidence, even at the risk of repetition. The basis for the idea lies primarily in the large body of evidence establishing the relative immobility of chromosomes during the resting stage. The aggregation of the kinetochores close to the division center in late anaphase brings the chromosome arms into a parallel or radiating arrangement. The closing in of the new nuclear membrane might then tend to bring the ends together. Granted relative immobility through the resting stage, an approximation of the bouquet arrangement would thus be already determined by the preceding telophase orientation.

The evidence against this interpretation of the bouquet has been presented by Schrader (1941b); I will summarize it here. First, telophasic reorganization will tend to bring chromosome ends together only if the kinetochore is median or nearly so. The formation of a typical leptotene bouquet in Phrynotettix (Wenrich, 1916) whose chromosomes have effectively terminal kinetochores thus shows that half of the chromosome ends have moved through the nucleus and oriented at the pole opposite that approached at telophase. Again, the hypothesis would demand a definite and fairly uniform chromosome length. In Choeradodis, with marked size differences among its chromosomes, the leptotene bouquet shows the kinetochore of the shortest element to be relatively close to the pole at which the ends are aggregated, while in only the longest chromosomes with median kinetochores do the latter retain their telophasic position. Third, typical bouquets occur in several Hemiptera (Geitler, 1937; Schrader, 1941b) although a kinetochore-center telophase aggregation cannot here—due to the diffuse nature of the kinetochore—be a causal factor (see, however, some contrary evidence in Ris, 1942). The converse of this argument is also applicable: many forms with pronounced aggregation of kinetochores at the center in telophase fail to show any bouquet stage in the succeeding meiotic prophase.

Again, we are confronted in many cases with an apparent shift in the spatial relations of center and kinetochores between telophase and meiotic prophase. Thus in Locusta (Mohr, 1916) the median kinetochores are aggregated near the center in telophase but prophase shows them at the opposite side while the chromosome ends are now grouped at the center. None of the suggestions seeking to reconcile these conditions independently of active chromosome orientation, such as a rotation of the nuclear contents through 180° (Janssens, 1924; Geitler, 1934) or a migration of the center through a similar arc (Schreiner and Schreiner, 1906; Gelei, 1921), meets the basic objection that polarization in the bouquet involves a more precise orientation and pronounced focussing of ends to a single circumscribed region than any telophasic orientation and reorganization would entail.

The formation of a typical bouquet in late prophase, following a completely non-polarized stage such as occurs in certain of the mantids, proves the meiotic polarization to be a distinct process involving forces not operative in relic orientation. The mantid evidence is valuable for its clarity rather than its novelty. Similar evidence has been available since 1921 in Gelei's careful analysis of the formation of the leptotene bouquet in Dendrocoelum oöcytes. He was able to count close to the total number of chromosome ends in the early pre-bouquet leptotene nucleus, and found them distributed, peripherally, but at random relative to the division center, throughout the nucleus.

Finally, the evidence just presented although sufficiently conclusive in itself, is secondary to the basic fact now also well established that bouquet formation involves a special activity of chromosome ends, center, and nuclear membrane.

The action of the chromosome ends in polarization is clearly evident in bouquet formation in mantids, as in many other organisms. Gelei (1921) was able to follow the movement of the knobbed ends of the Dendrocoelum chromosomes as they converged from a random distribution to aggregate in a plate-like cluster on the nuclear membrane underlying the center. The same process occurs in the formation of the second bouquet in certain mantid species. Furthermore, the variation in timing of polarization relative to degree of chromosome contraction seen in Paratenodera provides transitional stages between polarizations in which ends only are active, and those in which the whole compact body of the chromosome seems to be affected. This suggests that in the latter type, also, a special activity of ends may be involved, and adds to the growing body of evidence bespeaking special functions in these structures.

Action of the center in determining the pole of the bouquet has also long been recognized (Buchner, 1910; Ahrens, 1936, and others). It is strikingly evident in those mantid nuclei in which a single or monopolar bouquet first forms at the still undivided center and is then transformed into a double, bipolar, bouquet as the daughter centers move to opposite sides of the nucleus accompanied each by a group of chromosome ends on the inside of the membrane.

That the nuclear membrane takes a definite, if still undefined, part in polarization is becoming increasingly clear. Schrader (1941a) has shown that it plays more than a passive role in the movements of the centers as well as of the chromosomes. In the mantids the close application of terminal chromomeres to nuclear membrane, and the abrupt cessation of polarization on the collapse of the membrane, further support the thesis.

In conclusion: it appears definitely established that true bouquet formation is a special process, basically distinct from relic orientation, involving the operation of forces not active in the latter, and dependent on special activities of chromosome ends, centers, and nuclear membrane. Bouquet formation while widespread, is not, however, a universal nor essential element in meiosis. Its adaptive significance when, as is usually the case, it occurs prior to synapsis, lies in facilitating chromosome pairing, and was early recognized (v. Kemnitz, 1913; Gelei, 1921). The second bouquet stage, however, can have no such value since it occurs in post-synaptic stages. It introduces a maneuvre not ordinarily encompassed in the meiotic cycle, and one which shows the complexity of cyclical changes which may be involved in the various constituent processes of mitosis. Whatever its utility to the species, it emphasizes once again the amazing range of possible variations in the interplay of different factors in normal meiotic mechanisms.

II. Kinetochore Movements

a. Initial separation of kinetochores.

First of the several striking kinetochore movements during the meiotic prophase is the initial separation of homologous kinetochores. The first opening between the chromosomes of a bivalent is localized at the region of the kinetochores and is strongly suggestive of repulsion between these bodies. Swanson (1942) questions the efficacy of the kinetochore as a repelling body, and holds that the marked separation of kinetochores and the attenuation of the chromosome between kinetochore and nearest chiasma, usually attributed to such repulsion, is more probably due to a poleward "pull" of the spindle. Although this position appears well taken in Tradescantia, it is untenable in the mantids. Here in several species, separation of homologous chromosomes is at first limited to the kinetochore region, and occurs while the nuclear membrane is still intact. Furthermore the plane of kinetochore separation bears no relation to the position of the centers nor the future spindle axis.

Neither can we ascribe kinetochore separation to despiralization, although the fact that the latter is initiated at the kinetochore region and proceeds distally makes the assumption at first thought credible. Against it is the fact that there is no constant correlation between time of kinetochore separation and phase of spiralization cycle in the different species. Thus in the two geographic types of *Stagmomantis carolina* kinetochore separation starts at widely different points in the spiralization cycle, and in Paratenodera the bivalent has reached a contraction approximating that of metaphase before the kinetochores separate.

Again, in Callimantis (Hughes-Schrader, 1943) the differential behavior of kinetochores in quadrivalents and in bivalents shows the degree of separation to increase with the number of kinetochores present, other factors being equal. Of course, in this case, some special activity of the half spindle components, independent of the growth of the spindle as a whole, cannot be excluded, but the data seem more simply explicable on the hypothesis of kinetochore repulsion.

Thus in mantids all available evidence points to an autonomy of the kinetochores in their initial separation; the force involved is localized in or operates through the kinetochores themselves. While not establishing the nature of the force involved, the data are in harmony with the concept of a mutual repulsion between homologous kinetochores at certain stages of their cycle.

b. The stretch phenomenon.

A second and unique phase of kinetochore activity is initiated when, on the breakdown of the nuclear membrane and the formation of the spindle in the nuclear area, homologous kinetochores move suddenly toward opposite poles forcibly stretching open the bivalent between them. In bivalents with no previous repulsion of kinetochores, their separation and bipolar orientation is simultaneous; where repulsion precedes spindle formation, the kinetochores shift their position so that one points toward each pole. Obviously the force involved is operating through the kinetochores. White (1941) suggests that as the spindle forms the kinetochores become attached to it, and the spindle then elongates carrying the kinetochores toward the poles and stretching the chromosomes.

An analysis of spindle elongation and poleward movement of kinetochores is given in Table I. Spindle elongation is computed from the relative distances between the centers at the different stages; kinetochore movement similarly, in terms of inter-kinetochore distance in the longest rod shaped bivalent. Measurements are in ocular micrometer units, and each figure represents the average of some ten measurements. A correlation between spindle elongation and kinetochore movement is evident; Liturgousa and the Virginia Stagmomantis, with the greatest increase in spindle length, show the greatest kinetochore movement, while Choeradodis with the least spindle elongation has the slightest.

Despite this, spindle elongation is definitely not the only factor in the poleward movement of the kinetochores. In all but one of the five types the increase in spindle length is definitely less than the distance travelled by the kinetochores (compare columns 6 and 10 of Table I). Thus in the Virginia Stagmomantis only 5.2 units out of 8.5 may be attributed to spindle elongation; in Choeradodis only 2.4 units out of 5.1. The former case is particularly significant since chromosome length, which will be a factor in interspecific differences in degree of kinetochore separation, is the same in the two types of Stagmomantis.

Another line of evidence also demonstrates the existence of a second factor in the poleward movement of the kinetochores. It will be noted that in each case the elongation of the spindle follows quickly on its formation; thereafter spindle length remains constant or even gradually decreases up to final metaphase (Table I). The asynchrony of the bivalents in the kinetochore movement is so great that in every case the maximum spindle length is attained before all of the bivalents have opened. Since all bivalents are eventually stretched open, it is clear that in many cases the poleward movement of the kinetochores is independent of spindle elongation.

What is the nature of this second factor in the kinetochore movement? It may be simply a continuation of the mutual repulsion of homologous kinetochores already apparent in some species during the preceding stage. The orientation of the bivalent and the consequent direction of kinetochore movement would then be determined by the longitudinal structure of the spindle, permitting repulsion to act in the longitudinal axis while blocking it in any other plane. An alternative possibility exists; an attraction between center and kinetochore may come into play on the breakdown of the membrane and the formation of the spindle. Such attraction is indeed suggested by the random orientation of the kinetochores in the sex trivalent which move toward the nearer pole irrespective of their homology. The terminal alignment of the sex chromosome, completed before the poleward movement of the kinetochores begins, may result in too great a separation of their kinetochores for repulsion to be effective and thus allow the attraction between center and kinetochore to be visibly expressed.

It must not be forgotten, moreover, that half spindle components form between kinetochores and center at the time these movements are taking place. Some activity on their part, independent of the elongation of the spindle as a whole, cannot be excluded. Thus while the nature of the forces involved cannot yet be determined, the data do demonstrate that the poleward movement of kinetochores and resultant stretching of the chromosomes are due to more than one agency, and involve in addition to spindle elongation, other factors which may include repulsion of homologous kinetochores, kinetochore-center attraction, and possibly some activity of half spindle components.

c. Reapproach of homologous kinetochores.

On the completion of the poleward movement of the kinetochores, a precisely opposite action ensues. Homologous kinetochores re-approach each other and in so doing move away from the poles. The extent of this movements varies in the different species (last column of Table I). The causal factor seems to be the resumption of the normal coiling process, interrupted and partially undone by the preceding stretching.

d. Metaphase orientation.

The gradual movement of the chromosomes toward the equator of the spindle follows—and largely overlaps in time—the re-approach of the homologous kinetochores. It may even overlap the preceding stage of poleward kinetochore movement. It follows from this asynchrony that some kinetochores are still moving toward the poles while others are moving away from them, and that movement of the whole bivalent toward the equator may be superimposed on one or both of the others. The hypothesis that equatorial orientation is caused by repulsion between kinetochore and center is thus either untenable or requires the subsidiary assumption of a reversal of charge in the kinetochores—a reversal occurring, moreover, at different times in different bivalents. Again, since the spindle is completed long before movement of chromosomes to the equator begins, the latter cannot be attributed to ingrowth of spindle fibers from the poles pushing the scattered chromosomes to the equator. Finally, since both the re-approach of homologous kinetochores and the movement to the equator occur subsequent to the formation of half spindle components, a considerable elasticity in the action of these elements is indicated.

During the movement to the equator the bivalents shift position as wholes retaining their bipolar orientation. It is thus impossible to tell whether the movement is dependent on kinetochore action, as is well established in other forms. In the sex trivalent, however, the kinetochores definitely take the lead in metaphase orientation. In the re-orientation of its three elements the kinetochores alone are active while the arms appear relaxed. As to what force or forces underlie the movement to the equator and the co-orientation of homologous kinetochores, the present data give no clue.

III. Chiasmata and bivalent structure

Cases already on record demonstrate that chaismata are not the sole, nor an essential, factor in maintaining the meiotic association of homologous chromosomes in late prophase and metaphase (references in Hughes-Schrader, 1943).

Bivalents clearly devoid of chiasmata may retain the parallel association of their chromatids. Terminal connections between homologues at metaphase may be formed in complete independence of previous chiasma formation. We may take it as established that different species vary in the factors involved in the later stages of the meiotic association of chromosomes.

The present study shows further that (1) such variation exists even among closely related species, and (2) that bivalents within a single species may similarly vary in the factors determining the association of their homologues. White (1941) was unwilling on the evidence then available to admit such variation in a basic meiotic mechanism in so closely related a group of species as the mantids. Confronted on the one hand with the absence of chiasmata in the bivalents of Callimantis, and on the other with contrary although indirect evidence in all other species studied, he holds that the differences are superficial only. This implies either that the indirect evidence for chiasmata in the majority of species will prove unfounded or, as White further suggested, that chiasmata may be present in the Callimantis bivalent, but, in the absence of the stretch phenomenon in that species, are not disclosed due to the close parallel association of homologous chromosomes up to anaphase.

As to the first alternative, the present study offers strong presumptive evidence for the occurrence of chiasmata in certain bivalents of some species. The evidence lies in the relatively high frequency of open cross formations in opening bivalents, in which the association of non-sister chromatids distal to the opening of the cross may be clearly demonstrated. That such configurations necessarily imply previous chiasmata cannot be taken as completely proved (contrary evidence in the somatic chiasmata of Drosophila ganglion cells, Kaufmann, 1934), but the weight of evidence from many sources (heteromorphic bivalents, interlocking, etc.) is certainly in favor of this interpretation.

The second alternative is definitely untenable in the light of the evidence now available. Re-investigation of Callimantis (Hughes-Schrader, 1943) has confirmed White's (1938) earlier conclusion that chiasmata are absent. Neither in the parallel association of four chromatids in the prometaphase and metaphase bivalent nor in its anaphasic disjunction are chiasmata involved. The present study offers additional evidence that the Callimantis type of bivalent on the one hand, and the rod and ring types of other mantids on the other, present real differences in structure and in the forces underlying the association of the homologues. The differences in the metaphase form of bivalents are not ascribable simply to presence or absence of the stretch phenomenon. Thus we have seen in Liturgousa one bivalent of the Callimantis type, subjected to the same stretching that results in rod and ring formation by the other bivalents, open out without terminal connections and on re-contraction resume the parallel association of its homologues. Conversely, in Choeradodis, where the stretching of the bivalents on the spindle is almost nil, rod and ring types are nevertheless produced. In view of these lines of evidence the conclusion is justified that even closely allied species vary in the factors underlying the late meiotic association of homologous chromosomes.

Within the single species, also, the presence of more than one factor is demonstrable. Thus in the case of Liturgousa cited above, factors other than chiasmata determine the association in one bivalent, while others as consistently disclose the open cross with its implication of previous chiasmata. In Choeradodis, also, diversity of factors is evident.

Three separable factors in the late meiotic association of homologous chromosomes can be distinguished. They may operate separately or in various combinations in bivalents of different species and in different bivalents of one species. The first of these factors—the pairing force independent of chiasmata which we must assume determines the persistent parallel association of chromatids in the Callimantis type of bivalent—I shall term, for brevity in discussion, lateral attraction. The second, expressed in the formation of terminal connections between separating homologues, is recognized as terminal attraction. The term attraction is here used in a purely descriptive sense, without implication of the nature of the force involved. The third factor is the action of chiasmata. Let us consider these factors, as expressed in the structure of the mantid bivalents, separately.

Lateral attraction is demonstrated most clearly in the Callimantis bivalents where the four distinctly separated chromatids of each arm maintain their parallel association, without chiasmata, until anaphase. It is similarly expressed in the single Callimantis-like bivalent of Liturgousa, and in several of the Choeradodis bivalents whose homologous chromosomes are clearly separated but parallelly associated until stretched apart on the spindle. The data of the present study further demonstrate that lateral attraction may be variously localized in the individual bivalent. This is seen in those bivalents of Choeradodis whose homologous chromosomes show no contact during the pseudo-diakinetic period and where chiasmata are thus excluded as a factor in the association. Of these bivalents some show the chromosomes parallelly paired and equidistant along their whole length. In others the pairing segment is reduced to a short region, variously localized in different bivalents, with the homologues flaring widely apart elsewhere. A similar differential localization of lateral attraction is apparent in the diakinetic bivalents of the egg of the grass mite, *Pediculopsis graminum* (Cooper, 1939).

Terminal attraction is expressed in the resistance of apposed chromosome ends to the forces of anaphasic separation in the bivalents of Callimantis. In this case no fusion or physical connection of any kind between the chromatid ends is apparent. In the other mantid species the operation of terminal attraction is accompanied by the formation of persistent terminal connections between the ends of homologous chromosomes during the stretching of the bivalents on the spindle. So extreme is the tension produced by the movement to opposite poles of homologous kinetochores that the chromosomes are often attenuated to thin threads, and yet the terminal connections persist. In these cases, unlike Callimantis, a real fusion of certain constituents of the chromosome is suggested.

Terminal attraction may operate quite independently of chiasmata, as is shown in Callimantis and in other cases on record, perhaps most strikingly in Rhytidolomia (Schrader, 1940). This independence is also demonstrable in Choeradodis. Here several bivalents are clearly devoid of chiasmata during the pseudo-diakinetic period. Yet in the majority of nuclei at final metaphase all bivalents are of either the rod or ring type, with one or two terminal connections respectively. Obviously in certain bivalents terminal connections have formed independently of the terminalization of chiasmata.

Chiasmata are absent in late prophase and metaphase in many mantid bivalents and even when present appear to play but a subsidiary role in meiotic association. Since "repulsion" between pairs of chromatids, so diagnostic a feature of diplotene and diakinesis in most forms, is not obligatory in mantids, chiasmata when present will not be expected greatly to modify bivalent form in prophase. Nor is the form of the metaphase bivalent dependent on chiasmata to the extent which is usually assumed. We have seen that terminal connections between homologous chromosomes cannot be interpreted as invariably the sequelae of chiasma terminalization. They may also result, as is the case in certain Choeradodis bivalents, simply from terminal attraction between those ends which were held together earlier by lateral attraction and were finally brought into contact by the stretching of the bivalent. When chiasmata are present, their terminalization may well have a similar effect; it will bring chromatid ends into contact and thus possibly facilitate the operation of terminal attraction and the formation of terminal connections.

The nature of the forces involved in lateral and terminal attraction is obscure. The formation of a non-staining pellicle common to both homologues has been suggested, but no positive evidence of its existence is available. The intense staining of terminal connections in many mantid bivalents suggests a fusion of some chromosome constituent. But it must be remembered that in certain cases of terminal attraction in other forms (e.g. Rhytidolomia, Schrader, 1940) the chromosome ends move toward each other over a considerable distance and any fusion that may occur is thus secondary to the initial attraction. The same holds true for lateral attraction in the secondary metaphase pairing in spermatocytes of Lepidosiren (Agar, 1911), and in the re-association of previously separated chromatids in the second meiotic division of certain coccids (Hughes-Schrader, 1931).

Somatic pairing is similar in certain respects to the lateral attraction of meiosis. In this connection it is interesting that White (1938) finds no somatic pairing in Callimantis in which lateral attraction is so strongly expressed. Nor have I any convincing evidence of its occurrence in the species here reported, with the possible exception of Choeradodis spermatogonia, and even here the association of homologues is neither close nor constant. Oguma (1921) reports a similarly indefinite association in the spermatogonia of *Tenodera aridifolia*. Clearly there is no obligatory relation between somatic pairing and the lateral attraction of meiosis.

SUMMARY

1. *Bouquet formation.* An analysis of the second polarization or bouquet stage in the meiotic prophase of the males of several species of mantids shows bouquet formation to be a special process, basically distinct from the relic or Rabl orientation, and involving special activities of chromosome ends, division centers, and nuclear membrane.

2. *Kinetochore movements.* (a) The initial separation or "repulsion" of the homologous kinetochores in the meiotic bivalent is shown in several species to be independent of division centers and spindle and appears to be autonomous to the kinetochores. (b) The pre-metaphase poleward movement of kinetochores and consequent stretching of the meiotic chromosomes are in part due to the

elongation of the spindle, and in part to another factor or factors which may include kinetochore repulsion, kinetochore-center attraction, and a special activity of the half spindle components. (c) Resumption of coiling after the pre-metaphase stretch results in the re-approach of the homologous kinetochores and their movement away from the poles. (d) Movement of chromosomes to the equator regularly overlaps the movement (c) above, and may overlap (b), thus excluding any hypothesis of metaphase plate formation in which the chromosomes are regarded as passive.

3. *Chiasmata and bivalent structure.* Three separable factors in the late meiotic association of homologous chromosomes can be distinguished: (a) lateral attraction, which is independent of chiasmata and is variously localized in different bivalents; (b) terminal attraction which operates in some bivalents quite independently of chiasmata, and in others follows chiasma terminalization; and (c) the action of chiasmata. Absent in late prophase and metaphase in certain bivalents, the presence of chiasmata is inferred in others from the frequency of open cross configurations. These three factors may act more or less separately, and in various combinations, even in closely allied species, and in different bivalents of a single species.

4. Males of *Stagmomantis carolina* from Virginia and from Barro Colorado Island, C. Z., identical taxonomically and in chromosome complement, differ in the time of spindle formation relative to the stage of bivalent development in the meiotic prophase.

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